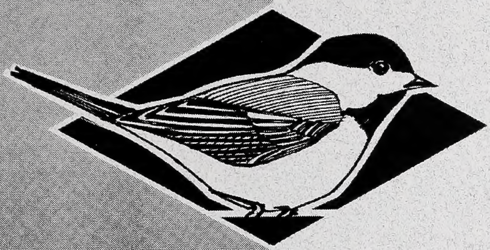

Fish & Wildlife Division

RESOURCE STATUS AND ASSESSMENT BRANCH

Northern Leopard Frog Reintroduction

Raven River –Year 2 (2000)

Alberta Species at Risk Report No. 13

Alberta
SUSTAINABLE RESOURCE DEVELOPMENT

Northern Leopard Frog Reintroduction

Raven River – Year 2 (2000)

Kris Kendell

Alberta Species at Risk Report No. 13

September 2001

Project Partners:

Alberta
SUSTAINABLE RESOURCE DEVELOPMENT

Alberta Conservation Association

ALBERTA SPORT, RECREATION PARKS & WILDLIFE FOUNDATION
Enhancing Alberta's Communities

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My sincere apologies to anyone I have neglected.

This project was completed when the Fisheries and Wildlife Management Division was part of Alberta Environment; this division is now part of Alberta Sustainable Resource Development.

EXECUTIVE SUMMARY

The northern leopard frog (*Rana pipiens*) is designated as *Threatened* in the province under the Alberta Wildlife Act (Alberta Environment 1996) and is nationally listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as a *Special concern* species (COSEWIC 2000). The leopard frog has exhibited population declines and is currently extirpated from much of its former range in Alberta. The historic distribution of the leopard frog in Alberta is closely associated with major river drainages that may reflect optimal breeding and hibernation sites. Information on current known leopard frog populations indicate that it is largely extirpated from the central parkland and has virtually vanished from the North Saskatchewan River drainage basin and is completely absent from the upper Red Deer River drainage basin. Isolated and fragmented, remnant leopard frog breeding populations in southern Alberta are vulnerable to disturbance and degradation, potentially leading to further local loss of the species. The northern leopard frog has exhibited little ability for natural dispersal back into historic parts of its range.

A management decision was made in 1998 to begin a reintroduction project for the leopard frog. The goal of the project is to re-establish the leopard frog in historically occupied habitats in the headwaters of the upper Red Deer and North Saskatchewan River drainage basins, consequently allowing natural downstream dispersal along these watersheds.

The northern leopard frog conforms to many of the requirements proposed in conservation literature for successful translocations. In April 1999, a pilot captive rearing program was initiated at the Raven Brood Trout Station (Sustainable Resource Development) located southeast of Caroline, Alberta and in the upper Red Deer River drainage. The facility offers infrastructure, managed access, and two rearing ponds that provide a controlled environment in which leopard frogs can be reared and temporarily confined. Several release sites were chosen in the area proximal to the Raven Brood Trout Station based on historic leopard frog records and the presence of potential suitable breeding, summering and over-wintering habitat. A second year of managed captive rearing of leopard frogs was undertaken in 2000. To date, approximately 2500 juvenile leopard frogs have been captive reared and released into the wild near Caroline, Alberta. However, apart from a single observation in the spring of 2000 of a 1999 released frog, no released leopard frogs have been capture or observed since.

During the captive rearing process, water quality, growth and development of the tadpoles, natural history observations, and population numbers were recorded and monitored. Prior to release into the wild, a percentage of the captive reared juvenile leopard frogs were randomly weighed and measured. All captive reared leopard frogs were marked using a Visible Implant Elastomer (VIE) tagging system. The bio-compatible marker was located in the webbing between the toes of one of the hind feet of each young-of-the-year leopard frog, producing an unique color and foot combination. The marking technique allows for the long-term monitoring of released frogs and the evaluation of the survival success at each release site and in each year of release. The following report details the results of the 2000 captive-rearing program of the northern leopard frog reintroduction project.

1.0 INTRODUCTION

Once a common and widespread species throughout much of Canada, the northern leopard frog (*Rana pipiens*) has vanished or declined in much of its historic range in the west. The decline of the northern leopard frog began in the 1960s in eastern North America and spread westward reaching Alberta in the late 1970s (Roberts 1981, 1994), leaving only a handful of isolated and local breeding populations. Once widely distributed in Alberta (Figure 1A.), remnant breeding populations of leopard frogs primarily occur in southern portions of the province, with the greatest concentration in the southeast (Figure 1B.). Today the northern leopard frog is designated *Threatened* under the Alberta Wildlife Act (Alberta Environment 1996). As a result, *Rana pipiens* merits special management consideration regarding existing populations and the habitats in which it occurs.

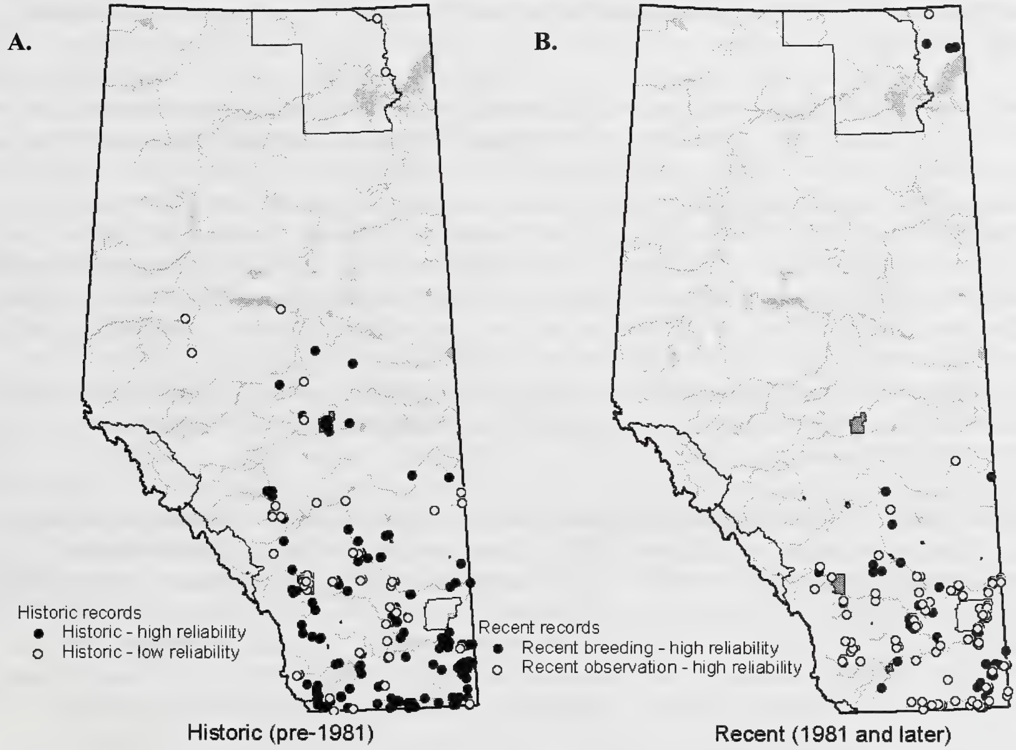

Figure 1. (A.) Historic (pre-1981) and (B.) recent (post 1981) records of the northern leopard frog in Alberta. (Details of these records are stored in the Biodiversity Species Observation Database (BSOD) and maintained by Alberta Sustainable Resource Development).

Amphibian populations are declining worldwide (Blaustein 1994, Pechmann *et al.* 1991, Wake 1991), however the extent of this phenomenon is not fully understood. For example, not all species of amphibians appear to be following this trend and a select few are expanding their ranges (Stebbins and Cohen 1995). In Alberta, leopard frog populations in some localities are in decline whereas the boreal chorus frog (*Pseudacris maculata*) and other amphibian populations sharing the same habitat remain healthy and abundant (Roberts 1994, Stebbins and Cohen 1995). It is generally believed that the contributing causes responsible for amphibian declines involve a variety of combined and unrelated factors. These include widespread atmospheric effects such as increased ultraviolet light, acid precipitation and global warming; climatic fluctuations such as droughts and cold; degeneration, fragmentation and loss of habitats; exploitation and over-collecting, disease and pathogens; effects of biocides such as herbicides and pesticides; intentional or accidental introduction of predators and/or competitors; and degradation of water and air quality. Drought, loss and degradation of habitat, disease and the effects of pollutants in waterbodies are just a few possible causes that may be responsible for the decline of leopard frogs in Alberta.

Remaining leopard frog populations are fragmented and isolated, making them vulnerable to disturbance, disease and natural disasters, which could potentially lead to further local extirpations. Because of fragmented populations that are separated by large areas of unsuitable habitat, the re-establishment of leopard frogs into historic areas may be dependent on transplanting individuals from existing major breeding populations in southern Alberta (Cottonwood Consultants 1986, Roberts 1987, Wershler 1991). In 1998, a management decision was made to initiate a reintroduction project involving the leopard frog. The primary goal of the reintroduction project is to re-establish the northern leopard frog to known historically occupied parts of its range that exhibit suitable over-wintering and breeding habitat components. The headwaters of the North Saskatchewan River and Red Deer River drainages were identified as the preferred release sites at which to establish breeding populations in hopes of natural downstream dispersal.

A pilot year and five individual studies preceded this year's program. The initial phase of the project began with a report by Fisher (1999). The report included an in-depth background investigation and literature review of the viability of translocating *Rana pipiens*. Fisher (1999) outlined a comparative evaluation and assessment of existing leopard frog populations and habitats in Alberta. Six populations of northern leopard frogs, identified by Wagner (1997) as inhabiting important breeding sites in the province, were selected in order to evaluate population demographics and measure ecological parameters to better understand winter physiology. In addition, eight potential translocation sites were selected in the headwaters of the North Saskatchewan and Red Deer River drainages. Water hardness, pH, alkalinity, temperature and dissolved oxygen were measured twice at potential translocation sites and on three occasions at currently occupied leopard frog sites. Fisher (1999) ultimately identified important candidate sites and source populations of leopard frogs for the proposed reintroduction project. Based on field observations and results, the Raven Brood Trout Station located southeast of Caroline, Alberta was selected as the preferred site for the reintroduction project.

A second report by Wendlandt (1999) investigated habitat preferences of the northern leopard frog and environmental conditions prior to and at the initiation of hibernation. Five leopard frogs

were fitted with radio transmitters and tracked using radiotelemetry equipment to potential hibernacula, at two sites in the Cypress Hills, in southern Alberta. In addition, the radio transmitters, transmitter harness design, fit and effectiveness were researched and then tested under field conditions.

A third report (Butterworth 1999) investigated the potential impact of diseases on the re-establishment of amphibians and the role of disease in amphibian declines. Butterworth (1999) also examined a protocol for the reintroduction of amphibians in order to prevent the introduction of diseases at sites of relocation and reviewed the amphibian diseases of importance to relocation programs in North America.

In April 1999, a pilot reintroduction program was initiated. Egg masses were collected from the Circle 'E' Ranch south of Bow City, in southeastern Alberta. The eggs were then transferred to two artificial outdoor rearing ponds at the Raven Brood Trout Station near Caroline, Alberta. Under controlled conditions, eggs and tadpoles were reared to the metamorph stage of development and released in suitable breeding and over-wintering habitats in the area immediately surrounding the trout station. Survival rate, development and dispersal were monitored throughout the rearing and release stages.

In September 1999, a fourth study investigating winter physiology and ecological requirements of the northern leopard frog took place in the area surrounding the Raven Brood Trout Station. The study involved the tracking of 16 translocated adult leopard frogs to potential and consequent over-wintering locations using radiotelemetry. The results of this study are detailed in Kendell (2000a).

A fifth water quality study relating to required aquatic over-wintering conditions for leopard frogs was undertaken in February and March 2000 near Brooks, Alberta. The purpose of the study was to collect a variety of ecological data on aquatic conditions necessary for leopard frog hibernation. A number of water quality parameter tests were conducted in nine study areas. All study areas were within the historic range of the leopard frog and contained one or more wetland sites. The study areas included sites where leopard frogs were extirpated, present or absent but the site could potentially support frog populations. Results of the study are detailed in Kendell (2000b).

2.0 STUDY AREA

The reintroduction site is located in an area proximate to the Raven Brood Trout Station, which is approximately 10 km southeast of the town of Caroline, Alberta, and immediately west of Highway 22 (Figure 2). Owned and operated by Alberta Environment, the Raven Brood Trout Station is situated on a quarter section of crown land.

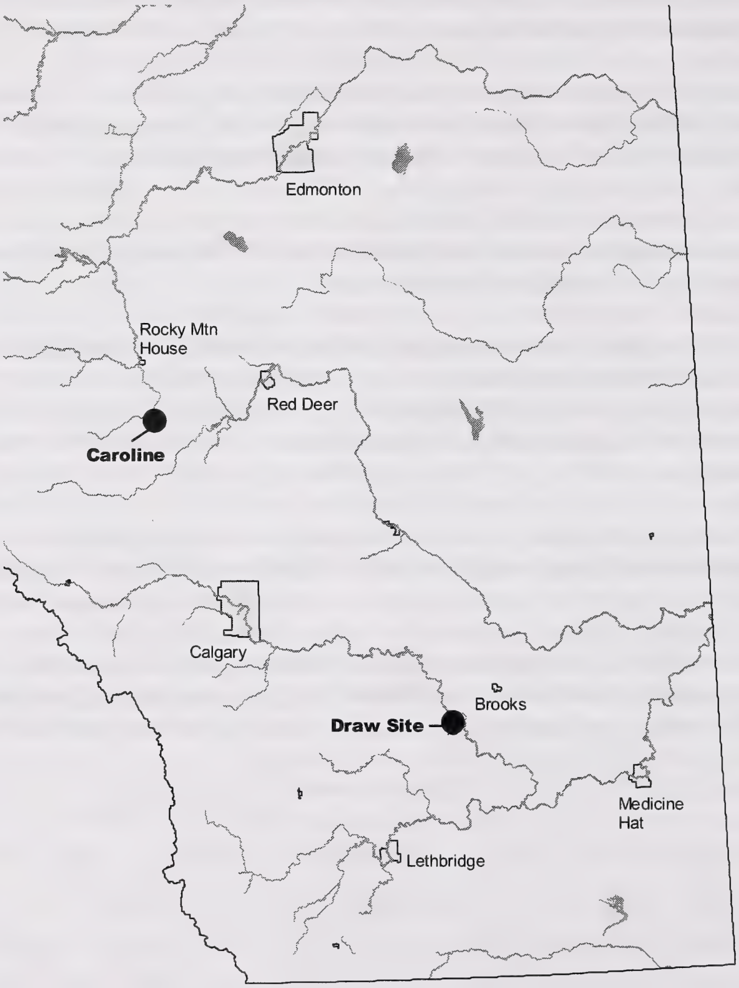

Figure 2. Map of Alberta (south of 55° latitude) showing the draw area in which egg masses were collected for the 2000 reintroduction program near Brooks, and captive rearing and release site located near Caroline.

Two confirmed leopard frog museum specimens (dated 1951 and 1955) are linked to the Raven Brood Trout Station and area (Wagner 1997) and are currently held at the University of Alberta Museum of Zoology in Edmonton, Alberta. The second record consisted of a series of late stage tadpoles and recent metamorphs (Biodiversity Species Observation Database (BSOD) 2000). According to information held in BSOD, leopard frogs were also observed in oxbows and upland wetlands in the vicinity of the trout station.

2.1 Raven Trout Brood Station Facility

The Raven Brood Trout Station offers a controlled amphibian rearing site in the form of two large outdoor ponds (Figure 3) that were formally used as trout raceways. These artificial ponds (hereafter referred to as west and east rearing ponds) offer managed access, water control structures and a means of temporary containment of captive-reared juvenile leopard frogs prior to being released into the wild. The ponds also provide an opportunity for a variety of controlled experiments and could serve as sites for a captive-breeding program.

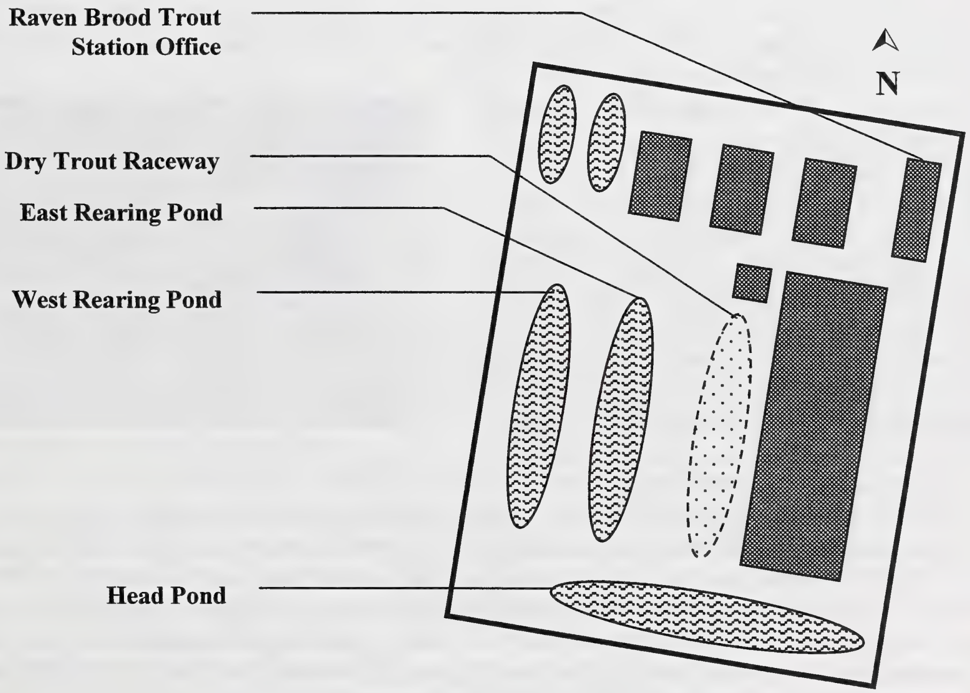

Figure 3. Line drawing of Raven Brood Trout Station, including rearing ponds, various buildings and other structures.

2.2 Rearing Ponds

The two rearing ponds are approximately 55 m in length and are capped with concrete walls at the south and north end with provisions for water inflow and outflow, respectively (Photo 1).

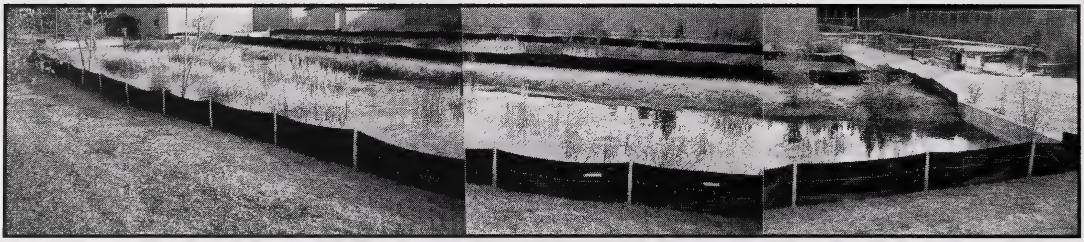

Photo 1. Leopard frog rearing ponds formally used as trout raceways at the Raven Brood Trout Station. Silt fencing surrounds each pond to control dispersal of captive-reared frogs (west rearing pond in foreground). Head pond can be seen at the top right of photo.

The maximum width of each elliptically shaped pond is approximately 7 m at the south and north ends and approximately 12 m at the centers. The bottoms of both ponds slope gradually from the water in-take end (south end) to the water outflow end (north end). A portion of a natural spring near the trout station is diverted into a primary head pond (Photo 2) situated just south of the two rearing ponds (Figure 3). From here, the spring water is further diverted and supplies the facility with water for its operations and the two rearing ponds. Water is supplied to each pond through large gate valves located in the head pond and ultimately enters each pond on the south end via an underground culvert. The north end of each rearing pond is fashioned with two 8 cm diameter gate valves that control the outflow of water. Each pair of valves is positioned 20 cm off the bottom of the respective ponds. Consequently, the north ends of the ponds can be drained to a minimum depth of about 20 cm, leaving the south ends nearly dry. The maximum depth of each pond is approximately 73 cm at the north end and 39 cm and 57 cm at the south end of the east and west rearing pond, respectively. One of the two valves on each pond is fitted with a stand pipe or overflow pipe that ensures a constant depth. The lengths of these standpipes can be manipulated to control and safeguard water depth in each pond. The two ponds are completely surrounded by silt fencing approximately 60 cm in height and are supported by rebar reinforced 2x2 pine stakes, spaced approximately 2 m apart. The distance between the silt fence and the waterline varies, however a minimum and maximum distance can be estimated at 0.5 m to over 2 m, respectively.

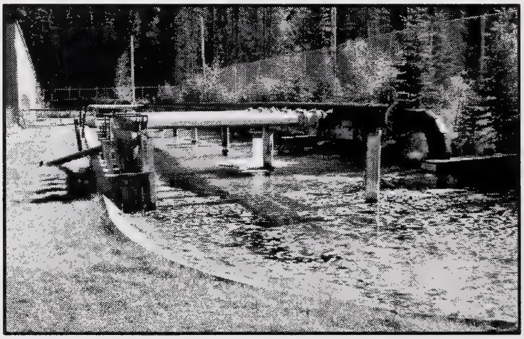

Photo 2. Head pond.

The substrate in the rearing ponds consists of gravel and cobble, with an organic layer of debris material. There is an abundance of both emergent (cattails, sedges, grasses) and aquatic plants in both ponds. The immediate shoreline consists of gravel, which is soon replaced by grasses and other small plants further upland. Several small shrubs and trees have also invaded the shores of each pond.

3.0 METHODS

3.1 Draw Sites

Spawning sites were identified in two of the three draw sites by conducting call surveys in known leopard frog habitat between sunset and approximately 2:00 am. Locations of calling leopard frogs were noted during the call surveys and egg mass searches were conducted the following day during daylight hours. Call surveys were not conducted at draw site 3; however, egg masses were located visually by searching suitable breeding habitat. In total, four leopard frog egg masses were collected from 3 different sites in southern Alberta near Bow City (Figure 4).

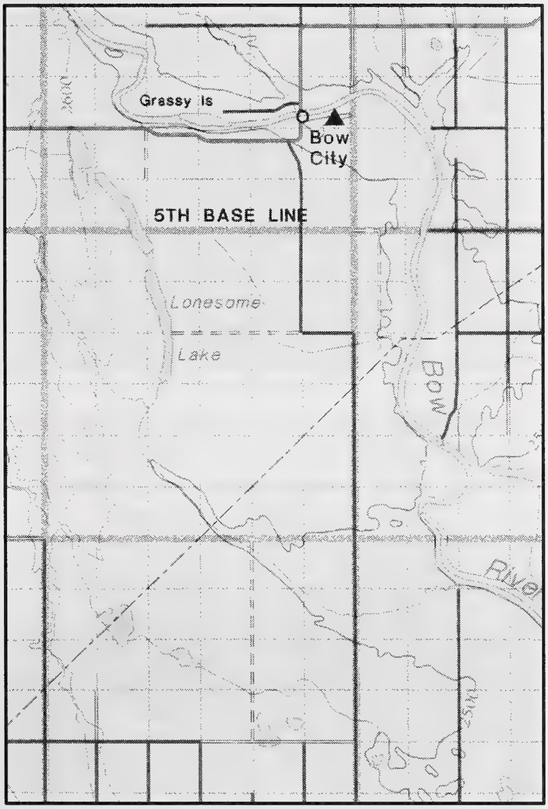

Figure 4. (Right) Topographical map showing area surveyed for leopard frog egg masses and breeding activity near Bow City, Alberta.

The draw sites for leopard frog egg masses collected in 2000 were identified and surveyed by Fisher (1999) and Wendlandt (1999) in 1998 and 1999, respectively. Between 4 May and 6 May 2000, the area surrounding Bow City was further surveyed and determined to be the most suitable area for the collection of egg masses for the reintroduction program in 2000. This was due in part to: 1) healthy leopard frog populations at a number of sites within the area, 2) broad occurrences of leopard frogs in the region, and 3) evidence of breeding activity over a widespread area. The three draw sites from which leopard frog eggs were collected were named draw site 1, 2 and 3. Efforts were made to avoid collecting egg masses from the draw sites utilized in 1999. However, one egg mass was collected from the same pond from which three egg masses were collected in 1999 (draw site 3). With the exemption of draw site 1, egg masses collected at each draw site accounted for less than 20% of the egg masses observed at that draw site on 6 May 2000.

3.2 Egg Mass Management

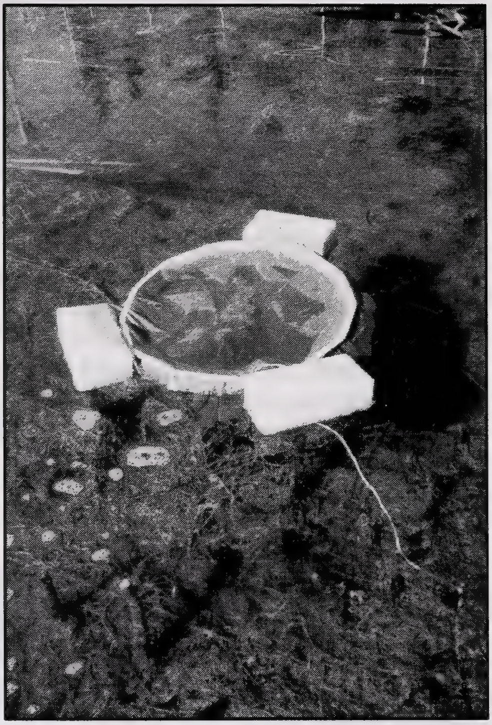

Photo 3. Egg mass predator exclosure floating in west rearing pond; note twine to prevent uncontrolled drifting.

Egg masses collected for the reintroduction were transported from the draw sites to the rearing site in a 2-litre thermos and a 4-litre cooler. Upon arrival the egg masses were subjected to a series of 20% water changes in their respective containers. Every 20 minutes, water from the rearing ponds was introduced to the transport containers until the water within the containers was completely replaced by water from the rearing site. In this way the egg masses were acclimatized to the rearing site's water temperature and chemistry in a slow and gradual manner, thus reducing potential water quality stresses to the egg masses and hatching tadpoles.

Once the acclimatization procedure was complete, each of the four egg masses was placed separately into a floating predator exclosure and placed in the east and west rearing ponds (Photo 3). The egg mass predator exclosure design for 2000 was an improvement over the previous year as it proved to be completely escape-proof and the finer and more flexible no-see-um mesh (versus window screen) eliminated injuries and trapping of tadpoles within folds and between the holes of the screen. The egg mass predator exclosure was designed to serve three purposes: 1) protect egg masses and hatchling tadpoles from aquatic invertebrate predators; 2) protect egg masses and hatchling tadpoles from avian predators and to a lesser degree terrestrial predators; and 3) provide a microclimate of warmer water for the development and growth of the eggs and hatchling tadpoles. The exclosures also allowed researchers to count the hatchling tadpoles prior to release into the rearing ponds.

In total, four predator exclosures were built (one for each egg mass). Materials incorporated into the predator exclosure design include # 500 ½ inch plastic shelf edge, 5-1/2 inch stainless steal hose clamps, no-see-um mesh, elastics, Styrofoam and twine. The dimensions of each exclosure were approximately 35 cm in diameter (frame) and 25 cm in depth (basket). The shallowness of the basket allowed the egg masses to be positioned near the surface of the water, where the maximum exposure to the warming rays of the sun and greatest concentration of oxygen (diffusion at the air-water interface) occurred (Photo 4). Two exclosures were placed in each of the two rearing ponds. Each exclosure was positioned in direct sunlight in the warmest ends of the ponds (south ends) and anchored in place by a short length of twine to minimize drifting (Photo 3). The twine had enough slack to allow some movement of the exclosure, to enhance water circulation and aeration around the egg masses and hatchling tadpoles.

Once the egg masses hatched, the hatchling tadpoles were confined to the predator exclosures until approximately one week after hatching. At the end of this holding period the tadpoles were free swimming and had completely consumed the jelly of their egg mass. The holding period resulted in hatchling tadpoles that were better able to avoid and react to potential aquatic invertebrate predators upon release from the floating exclosures and meant that the new tadpoles could travel to optimal sunning and feeding locations within the rearing ponds more efficiently. Recently hatched tadpoles confined to the predator exclosure fed on the egg mass jelly and micro-algae that grew on it. Later the hatchling tadpoles fed on abundant algae that grew on the large surface area of the no-see-um mesh as well as algae introduced into the predator exclosure by the researchers. Fine and delicate filamentous alga was selected as the premium source of supplemented food for the growing hatchling tadpoles. All tadpoles were counted using a plastic strainer (Photo 5) prior to release in each of the two ponds. Finally, the tadpoles were released into the same rearing pond in which their predator exclosures were placed.

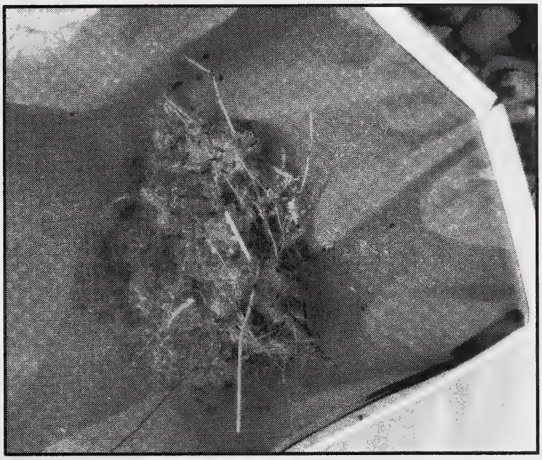

Photo 4. Leopard frog egg mass in predator exclosure. This egg mass was collected from draw site 2 and contained over 2900 eggs.

Photo 5. Tadpoles were counted individually using a plastic strainer.

Water depths in the two rearing ponds were maintained at their maximum levels in order to minimize water temperature fluctuations and achieve optimal water temperatures to promote the growth and development of the tadpoles (approximately 20°C). For the most part, the water in the rearing pond was stagnant with no in-or out-flow of spring water. Water lost through evaporation was replaced naturally through precipitation or, when needed, by the input of spring water from the head pond. When spring water was used to "top up" the rearing ponds, it was done slowly and gradually to prevent a drastic change in water quality and stress to the tadpoles.

3.3 Collection of Captive-Reared Leopard Frogs

Nets and specially designed funnel traps (Photo 6 and Photo 7) were used to capture young-of-the-year leopard frogs upon emergence and dispersal from the rearing ponds. The funnel traps were constructed out of 1/8 inch hardware cloth (funnels), duct-tape, 1½ inch PVC tubing (lead inside trap from funnel) and 70-litre plastic tote bins. A number of half inch diameter holes were drilled into each tote bin to provide adequate ventilation to prevent uncomfortable ambient temperatures for the trapped frogs and each trap contained roughly 1 inch of water so that the frogs would not desiccate. Traps were left unchecked for no more than 24 hours at a time and were often checked every few hours

Photo 6. (Above) Specially designed leopard frog funnel trap placed along silt fencing around edge of the east-rearing pond.

Photo 7. (Right) Two young of the year leopard frogs being funnelled into a funnel trap.

when they were being used. The weight, snout-went length (SVL) and physical abnormalities (i.e. missing limbs, wounds, etc.) of every tenth frog were recorded.

3.4 Marking

Captive-reared young of the year leopard frogs were marked using a Visible Implant Elastomer (VIE) tagging system (Northwest Marine Technology, Inc. 2000). The tagging system provided an externally visible internal identification marker. The marker consisted of a fluorescent elastomer agent, that when mixed with a curing agent, cures into a pliable solid at room temperature within 24 hours. This bio-compatible mark was injected using a 3/10 hypodermic syringe into the webbing between the fourth and fifth toe of the left hind foot of each young of the year leopard frog (Photo 8, A.-C.). A number of florescent elastomer colors are available, however green, orange and yellow elastomer was selected for injection in 2000. To facilitate ease of handling during the marking process, frogs were occasionally subjected to chilled (4-6 ^{o}C) spring water to reduce their activity level.

Unique colors and rear foot combinations were chosen for 2000 that were different from those of 1999. Color/rear foot combinations were chosen based on year of release and site released, within the reintroduction area. Frogs released at release site 1 (see section 4.5.1) were marked on their left foot with orange elastomer. Frogs released at release site 2 (see section 4.5.2) were marked with green elastomer on their left foot. Frogs held over winter in the west rearing pond were marked with yellow elastomer on their left foot.

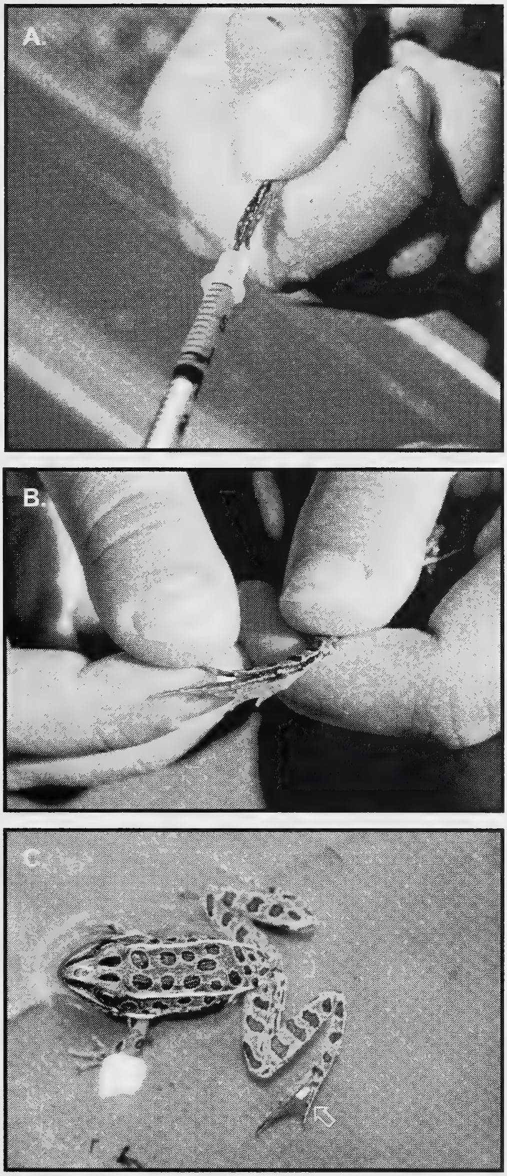

Photo 8. (right) Injecting a juvenile leopard frog with elastomer (A.) in the webbing between fourth and fifth toe of the hind foot (B.). A juvenile frog (C.) with a VIE mark on left hind foot (indicated by arrow).

3.5 Predator Control

Throughout the rearing process, efforts were made to reduce and eliminate the effects of potential and known predators on the tadpoles and young of the year frogs. Identified predators included predaceous diving beetle larva (*Dytiscidae sp.*), great blue heron (*Ardea herodias*), belted kingfisher (*Ceryle alcyon*), gray jay (*Perisoreus canadensis*) and mink (*Mustela vison*). To decrease the number of established aquatic predators in the west rearing pond, water levels were drawn down to nearly dry on 16 April 2000 and for several days thereafter. The east pond could not be given similar treatment because it was used to over-winter 1999 captive-reared juvenile frogs. Diving beetle larvae were captured using dip nets throughout the rearing process, netting was placed along the waterline of each rearing pond and scarecrows were erected on the shore to deter great blue herons and belted kingfishers utilizing the rearing ponds. Live traps were set and monitored by Fish and Wildlife staff near the rearing ponds to live capture and relocate problem mink.

Deceased young of the year leopard frogs discovered in the east and west rearing ponds were collected, bagged, labelled, placed into a freezer on site and later shipped frozen to the Canadian Co-operative Wildlife Health Centre in Saskatoon, Saskatchewan for necropsies and disease testing.

3.6 Release

Frogs were transported (< 25 minutes) to release sites within the reintroduction area using 70-litre tote bins. All frogs were released directly into suitable habitat, i.e. in abundant cover, on or along the shore of the selected waterbody. Both release sites selected for 2000, within the reintroduction area, were considered to possess suitable breeding habitat. Both summering and wintering habitat were present in the immediate area (see section 4.5). Release sites were monitored regularly after release, and post release surveys were conducted to determine dispersal activity of released frogs and to record other natural history observations or behaviour of the released frogs. Weights and SVL length measurements were taken on released frogs when they could be caught.

4.0 RESULTS

In total, 1277 young of the year leopard frogs were captive-reared, marked and released into the wild near the Raven Brood Trout Station, and 200 young of the year leopard frogs were detained in the west rearing pond at the facility to be over-wintered. Results collected in 2000 reflected the primary goal of the captive rearing program (i.e. producing the maximum number of healthy juvenile frogs) and were slightly different than those collected in 1999.

4.1 Draw Sites

In total, four leopard frog egg masses were collected on 6 May 2000 from three draw sites in southern Alberta, near Bow City. Two egg masses were collected from draw site 2 and one egg mass was collected from draw site 1 and draw site 3.

The four egg masses collected for the 2000 rearing season were, on average, similar in size to the three egg masses collected in 1999. The resulting number of hatchling tadpoles from each egg mass was also compared between years (Table 1). In total, 6692 hatchling tadpoles were counted from the four egg masses collected in 2000. The greatest number of eggs (estimated based on tadpoles counted) recorded over the two years of the reintroduction program occurred in 1999, with 3845 tadpoles being counted (information on the number that escaped or died during the hatch period is unknown). That egg mass measured 130x90x60 mm (Wendlandt and Takats 1999). The largest egg mass and highest number of tadpoles record in 2000 was obtained from an egg mass that measured 120x120x90 mm and hatched 2979 tadpoles (with only 17 mortalities during the hatch period and no escapees) (Table 1 and 2).

Table 1. Egg mass dimensions and tadpole numbers counted from each egg mass in 2000 and 1999.

2000 captive rearing program			**1999 captive rearing program**[†]		
Draw site / egg mass	Egg mass dimension (mm)*	Number of tadpoles counted	Draw site / egg mass	Egg mass dimension (mm)*	Number of tadpoles counted
Draw Site 1	90x80x70	1299	Draw Site 3	130x90x60	3845
Draw Site 2 (egg mass 1)	120x90x40	1994	Draw Site 3	100x80x60	2402
Draw Site 2 (egg mass 2)	150x120x90	2979	Draw Site 3	100x80x55	2045
Draw Site 3	**	420***			
	TOTAL	6692		TOTAL	8292

(*) Egg mass dimensions approximate
(**) Egg mass in process of hatching; exact measurements not possible at that time.
(***) Confirmed survived; an estimated 1500 hatchling tadpoles suffered mortality due to fungal infection.
(†) See Wendlandt and Takats 1999.

The hatching success was high in three of the four egg masses collected. The draw site 3 egg mass suffered the highest mortality between the egg and hatch stages of development and experienced an estimated 77.9% mortality, caused by what appeared to be a fungal infection (Table 2). Some of the increased mortality may be attributed to negative effects of transporting the egg mass while it was hatching. The dislodging of recently hatched tadpoles cannot be ruled out as one of the possible causes of the mortality observed. The recently hatched tadpoles may have also been more vulnerable to any water quality changes between the draw site and rearing pond once outside their protective egg jelly. The three egg masses collected at draw site 1 and draw site 2, exhibited very low mortality levels during the hatch period, ranging between

approximately 0.5% to 0.6% loss (Table 2). Overall, the percent of tadpoles that survived through metamorphosis was similar between 1999 and 2000, with a slight increase in survivor rate in 2000 (Table 3).

Table 2. Number of tadpole mortalities recorded after hatch from each egg mass collected in 2000 and prior to release into respective rearing ponds.

2000 captive rearing program			
Draw site / egg mass	Number of tadpoles counted	Number of mortalities recorded (% of total number of tadpoles per egg mass)	Number of days after complete hatch when tadpoles were counted
Draw Site 1	1299	6 (0.5%)	11 days
Draw Site 2 (egg mass 1)	1994	10 (0.5%)	10 days
Draw Site 2 (egg mass 2)	2979	18 (0.6%)	10 days
Draw Site 3	420	~1500* (78%)	0 days*
TOTAL	6692	~1534 (18%)	

(*) Draw Site 3 egg mass experienced significant mortality because of what appeared to be a fungal infection. As a result, healthy tadpoles were counted and removed from the predator exclosure on the date of complete hatch.

Table 3. Percent of tadpoles that survived to juvenile frogs in 2000 and 1999.

2000 captive rearing program		1999 captive rearing program	
Total number of tadpoles counted	6692	Total number of tadpoles counted	8292
Total number of YOY leopard frogs counted	1477	Total number of YOY leopard frogs counted	1430
Percent of tadpoles that survived to metamorphosis	22.1%	Percent of tadpoles that survived to metamorphosis	17.2%

4.2 Rearing Site

In contrast to 1999, tadpoles were not distributed equally between the two rearing ponds (Table 4). In order to minimize stress to developing leopard frog tadpoles (a frequent contributing factor to amphibian disease), contact with the developing tadpoles was restricted to initial tadpole counts upon release from the predator exclosures and final counts of metamorphosed young of the year frogs. Because a second acclimatization procedure would have been necessary if the tadpoles were to be divided evenly between the two rearing ponds, they were left in the ponds in which they hatched.

Table 4. Number of tadpoles released in east and west rearing pond in 2000 and 1999. The site from which they were collected denotes egg mass.

<table>
<tr><th colspan="3">2000 captive rearing program</th><th colspan="3">1999 captive rearing program</th></tr>
<tr><td rowspan="2">Draw site / egg mass</td><td colspan="2">Tadpoles Counted</td><td rowspan="2">Draw site / egg mass</td><td colspan="2">Tadpoles Counted</td></tr>
<tr><td>East rearing pond</td><td>West rearing pond</td><td>East rearing pond</td><td>West rearing pond</td></tr>
<tr><td>Draw Site 1</td><td>-</td><td>1299</td><td>Draw Site 3</td><td colspan="2" rowspan="4">8292</td></tr>
<tr><td>Draw Site 2 (egg mass 1)</td><td>2979</td><td>-</td><td>Draw Site 3</td></tr>
<tr><td>Draw Site 2 (egg mass 2)</td><td>1994</td><td></td><td>Draw Site 3</td></tr>
<tr><td>Draw Site 3</td><td>-</td><td>420</td><td>-</td></tr>
<tr><td>TOTAL</td><td>4973</td><td>1719</td><td>TOTAL</td><td>4146</td><td>4146</td></tr>
<tr><td>GRAND TOTAL</td><td colspan="2">6692</td><td>GRAND TOTAL</td><td colspan="2">8292</td></tr>
</table>

An uneven distribution of tadpoles between two rearing ponds with similar physical and chemical attributes allowed researchers to test the carrying capacity of the rearing ponds along with the health (vigour and robustness) of the captive reared froglets upon metamorphosis (see section 5.0).

The average weight and SVL of metamorphs (5-12 days after mass emergence) in 2000 were 5 g / 36 mm SVL (n=99) from the east rearing pond and 7 g / 41 mm SVL (n=42) from the west rearing pond. In 1999, the average weight and SVL of 178 juvenile frogs weighed and measured between 16 September to 23 September were 6.7 g / 36 mm SVL.

4.3 Water Quality

Water quality tests conducted included water temperatures, pH, and water hardness (KH and GH) (Table 5). The water in the rearing ponds was found to be relatively hard over the entire rearing period and frequently fluctuated. Conversely, pH was found to be fairly constant over the entire rearing period and was recorded as 6 between 17 May and 30 August. Water temperatures were recorded mid-day and steadily increased in response to increasing air temperatures over the summer. The accuracy and interpretation of the pH test used in 2000 was questioned when compared to the results of an alternate pH testing method. Therefore, the pH values taken in 2000 may be inaccurate and the actual pH may have been higher.

Table 5. Water quality recorded in east and west rearing ponds during 2000 rearing season between 17 May and 30 August.

Date	East rearing pond				West rearing pond			
	GH*	KH**	pH	H_2O °C	GH	KH	pH	H_2O °C
17 May	179	143.2	6	15	214.8	179	6	14
19 May	214.8	161.1	6	13	232.7	232.7	6	13
30 May	196.9	196.9	6	13	196.9	179.0	6	14
04 June	-	-	6	18	-	-	6	16
06 June	179	179	6	15	161.1	179	6	15
11 June	179	179	6	13	179	179	6	13
22 June	161.1	161.1	6	18	161.1	125.3	6	18
05 July	161.1	143.2	6	17	161.1	143.2	6	17
11 July	196.9	196.9	6	18	179	125.3	6	22
13 July	196.9	179	6	22	179	125.3	6	21
17 July	179	161.1	6	22	161.1	179	6	20
27 July	161.1	196.9	6	23	179	161.1	6	22
31 July	179	161.1	6	23	179	161.1	6	22
22 Aug	-	-	-	-	161.1	179	6	24
28 Aug	-	-	-	-	232.7	250.6	6	15.5
30 Aug	-	-	-	-	196.9	196.9	6	11

* (GH) = general hardness in ppm.
** (KH) = carbonate hardness (also known as alkalinity) in ppm.

4.4 Tadpole Development

A number of significant dates were noted during the development and metamorphosis periods of the egg masses collected in 2000. Egg mass deposition dates were estimated based on literature cited in conjunction with the time the eggs were collected from the draw sites (Table 6). The dates the egg masses began to hatch and completed hatching were also recorded. The egg mass collected from draw site 3 was in the process of hatching on 6 May and had the earliest hatch date, whereas the egg masses collected from draw site 1 and draw site 2 hatched on 16 May. The hatching period for the egg masses, with the exception of the egg mass collected draw site 3, lasted approximately four days. All egg masses had completely hatched by 19 May. Seven to 11 days after hatching, the tadpoles appeared to be active enough for release into the rearing ponds and without increased vulnerabilities to predators. The first true metamorph was not observed until 21 July in the west rearing pond. By 24 July, the first metamorph was observed in the east rearing pond. Mass emergence of young of the year began on 3 August and was complete by 8

August (Table 6). An unknown and comparably limited number of leopard frogs undoubtedly metamorphosed after 8 August from both rearing ponds.

Table 6. Significant dates during metamorphosis – 2000 captive rearing program. Egg masses are denoted by site from which they were collected.

Natural History Aspect	Date(s)
Egg mass deposited*	16 April – 30 April (Draw Site 3) 26 April – 05 May (Draw Site 2 – egg mass 1 & 2) 26 April – 05 May (Draw site 1)
Egg mass collection from draw site(s)	06 May 2000
Egg mass hatch date	
▪ Initiation	06 May (Draw Site 3) 16 May (Draw Site 2 – egg mass 1 & 2) 16 May (Draw Site 1)
▪ Complete	16 – 19 May (all egg masses)
Egg masses released from predator exclosure	19 May (Draw Site 3) 26 May (Draw Site 2 – egg mass 1 & 2) 30 May (Draw Site 1)
Metamorphosis/emergence**	
▪ Initiation	21 July (west pond) 24 July (east pond) Generally- 19 July to 26 July (both ponds)
▪ Mass emergence of YOY***	03 August (both ponds)

(*) Dates when egg masses were laid are estimated based on 1-3 week development period before hatching.
(**) Metamorphosis/emergence defined as YOY with all 4 limbs (with or without tail stub) and emerging from water to bask or breathe air.
(***) YOY = young of the year

known for each egg mass, it can be generally predicted based on field observations by a number of authors. It should be noted that the actual time required for hatching is dependent on water temperature.

4.5 Release (2000)

Leopard frogs reared in 2000 were released into the wild in two different locations near the Raven Brood Trout Station, referred to as the oxbow site and the cooling pond site. Both sites possessed potentially suitable breeding, summering and wintering habitat components.

In total, 1277 captive-reared frogs were released into the wild during the 2000 field season: 631 at the oxbow release site and 646 at the cooling pond release site. For the second consecutive year, a relatively small number of captive-reared young of the year leopard frogs were detained in one of the two rearing ponds for the purposes of a controlled over-wintering experiment. The west rearing pond was chosen for the second over-wintering attempt, as opposed to the east rearing pond that was used in 1999. This was because the west rearing pond's water control gate had been modified to eliminate and reduce water leakage. Two hundred juvenile frogs were marked with yellow elastomer in the left hind foot (Table 7) and released into the west rearing pond. Results of the over-wintering survival success of the detained frogs will be assessed in the spring of 2001.

Table 7. Summary of numbers of captive-reared leopard frogs (2000) released at each release site.

Release Site	**Numbers of YOY leopard frogs marked and released**
Oxbow Release Site	631
Cooling Pond Release Site	646
West Rearing Pond Release Site	200
TOTAL	1477

Marking of the frogs reared at the trout station commenced on 8 August, approximately 5 days after the mass emergence (metamorphosis) of the frogs in the rearing ponds. The bulk of the recently transformed young frogs were marked with elastomer between 8 August and 15 August (Table 8). By 15 August, 94.3% of the frogs had been marked and released into the two release sites.

Table 8. Dates captive-reared frogs were captured and marked with elastomer.

Capture/mark	**Date**
▪ Initiation	8 August
▪ Progress (% of total # of frogs processed – n=1477)	8 August – 15 August (94.3%) 16 August – 13 September (5.7%)
▪ Completion (% of total # of frogs processed – n=1477)	13 September (100%)

Because of the time and energy required to capture the remaining frogs and delayed metamorphosis by some tadpoles, marking and releasing of frogs continued through to 13 September. Between 16 August and 3 September the remaining frogs (5.7%) were captured and released into the release sites (Table 8).

4.5.1 Site 1 (oxbow release site)

The first area of release was located approximately 0.75 km north of the Raven Brood Trout Station and consists of a series of seasonal-to-permanent oxbows that occur primarily to the north and parallel to the Raven River, on pastureland (Figure 5).

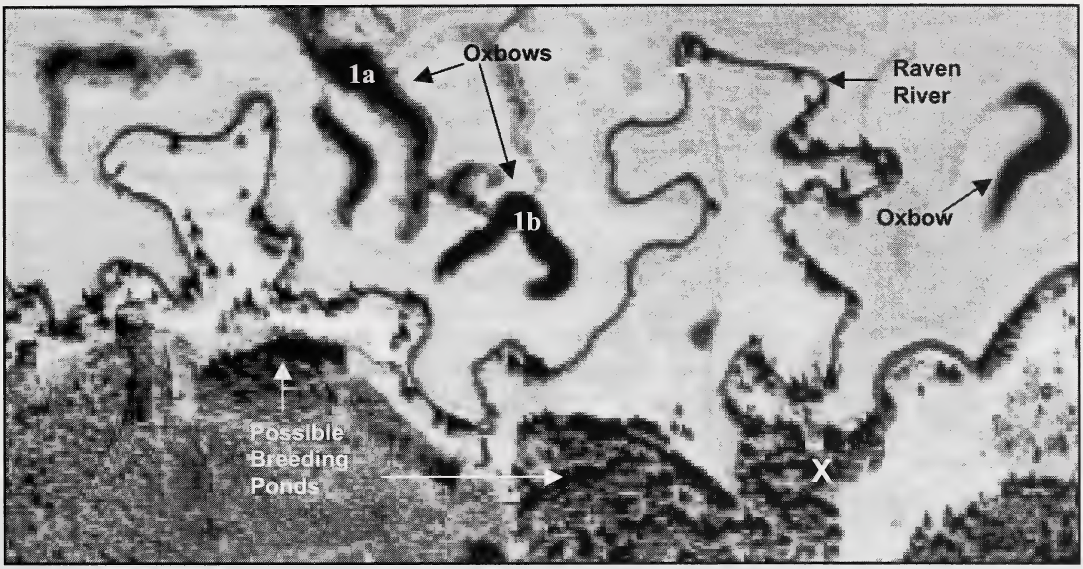

Figure 5. Oxbow release site located north of the Raven Brood Trout Station. The two oxbows in which frogs were released are labelled '1a' and '1b'. An 'X' indicates approximate confluence of Beaver Creek and the Raven River.

The oxbows are fed primarily by runoff from precipitation or snowmelt. Ground water or the water table may influence some of the oxbows that possess persistent and perennial moisture. The release site within the Oxbow/Raven River complex was selected based on field surveys in the fall of 1999 and spring/summer of 2000 regarding water depth and the permanence of a number of oxbows in the area (Kendell 2000a). Two oxbows were selected as the premium sites at which to release frogs because of their persistent water depth and evidence of successful breeding of other amphibian species, particularly wood frogs (*Rana sylvatica*) (Figure 5).

Alternate breeding habitat can be found south of the oxbow release sites and Raven River in small ponds created by tributaries of Beaver Creek. These tributaries are not influenced (or are insignificantly influenced) by spring water and therefore completely freeze in the winter. Although the Raven River offers the only potential over-wintering habitat in the vicinity of the release site (Figure 5), the proximity of these waterbodies should allow for a high percentage of released frogs to come into contact with it. No standing water of sufficient depth occurs in the immediate area that could be used by leopard frogs for hibernation.

The oxbows selected for release are situated on range land that largely consisted of grass species (*Agropyron* sp., *Poa* sp. etc.) and a variety of forbs such as common plantain (*Plantago major*), elephant's head (*Pediclularis groenlandica*), dandelion (*Taraxacum* sp.) and goldenrod (*Solidago* sp.). Small shrubs such as prickly rose (*Rosa acicularis*), snowberry (*Symphoricarpos occidentalis*) and silver willow *(Elaegnus commutata*) also occur in consistent patches over the area. Willow (*Salix* sp.) and a small number of aspen (*Populus tremuloides*) occur to the north and west along the edge of oxbow '1a' (see Figure 5). The majority of the oxbow ponds in the area, including the oxbows in which frogs were released, have a wide zone with thick emergent vegetation in the form of *Carex* sp. and grass species such as marsh reed grass (*Calamagrostis canadensis*). A variety of aquatic plants also occurs in the oxbows, including water-crowfoot (*Ranunculus* sp.). These habitat attributes provide abundant cover for tadpoles, frogs, and structures in which egg masses can be attached. Overall, the habitat structure and composition at the oxbow release site is consistent with known leopard frog summering habitat in the province and offers a wealth of food items, foraging area, cover and sunning locations.

4.5.2 Site 2 (cooling pond release site)

The second area of release was a large cooling pond located several meters to the south and west of the Raven Brood Trout Station (Figure 6). The cooling pond was selected as the second release site because of its persistence and suitability of breeding habitat. Over-wintering habitat, primarily in the form of springs and a spring fed creek, also occurred in the area to the east of the release site.

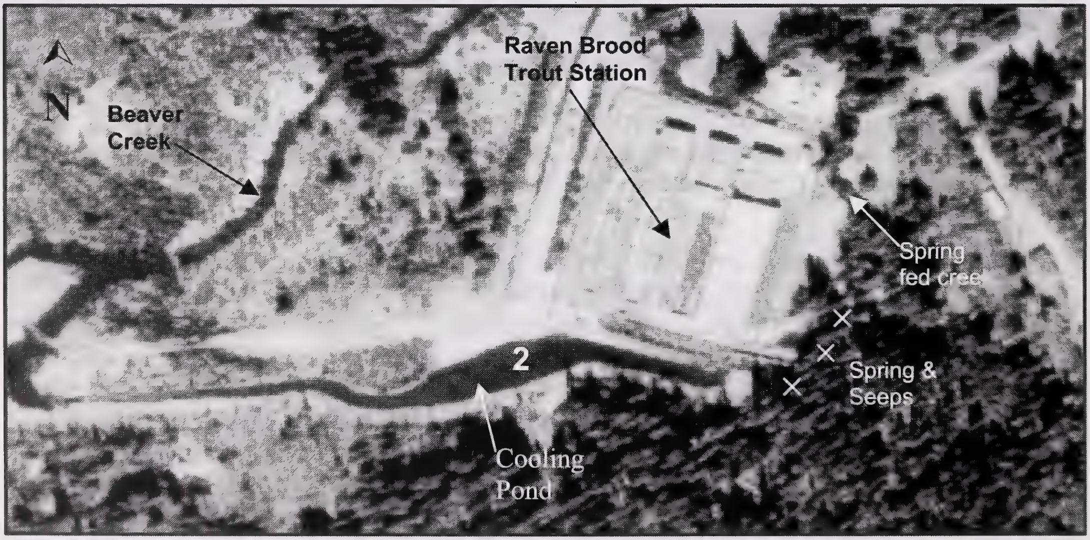

Figure 6. Cooling pond release site (2) and Raven Brood Trout Station.

The habitat surrounding the cooling pond release site is more treed than the oxbow site. A thick conifer forest consisting of pimarily white spruce (*Picea glauca*) occurs to the east and south of the cooling pond. The habitat to the northwest and west of the cooling pond is similar to leopard frog habitat found in the Cypress Hills in southeastern Alberta and consists of relativly open

stands of alders (*Alunus* sp.), willows (*Salix* sp.) and deciduous trees such as aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*).

The cooling pond itself is a relativly shallow waterbody with both open and heavily vegetated banks, offering good basking sites over much of the center portion of the pond. The east and especially the west end of the cooling pond, where it joins Beaver Creek, have abundant emergent vegetation appropriate for egg mass attachment and as cover for tadpoles and young frogs.

4.6 Release (1999)

Several informal surveys were conducted between the beginning of May and mid-July 2000 for leopard frogs reared in 1999. On 6 June, a crew of 10 individuals familiar with leopard frog natural history searched the 1999 release sites and surrounding area for leopard frogs. In total, 19.5 person hours were spent searching for leopard frogs on 6 June between 11:45 and 16:00. The survey was focussed on characteristic leopard frog habitat and along waterbodies or in wet areas. The survey was also conducted under ideal weather conditions. Air temperature averaged approximately 23°C and weather conditions were sunny with a relatively calm wind. The survey on 6 June resulted in one unconfirmed sighting of a single juvenile leopard frog from a reliable observer. Unfortunately, no specimen was photographed, collected or captured. A total of 81 wood frogs, 4 boreal toads and 2 boreal chorus frogs were observed during the 6 June survey. A number of informal surveys conducted between July 2000 and August 2000 also failed to record any leopard frog observations.

5.0 DISCUSSION

The practice of reintroducing endangered and threatened species in areas where they are currently extirpated has recently increased (Dodd and Seigel 1991) and is an accepted component of management and captive-propagation programs for endangered species (Conant 1988). The goal of these programs is to re-establish viable populations of species by increasing the number of sub-populations or increasing the number of individuals in small populations (Campbell 1980, Scott and Carpenter 1987), thus reducing the probability of extinction of the species in question.

Several experiments have suggested that the most effective way to rear frogs under artificial conditions is to start with egg masses procured in nature and carry the eggs and tadpoles through hatching and metamorphosis (Wright 1920). Traditionally, the rearing of frogs has been associated with the intensive commercial production of frogs as food animals and laboratory frogs for research and experimentation. Recently, it has been identified as a management technique to secure dwindling amphibian populations. Two major types of management systems have been used to rear leopard frogs from the egg stage and through transformation to juvenile frogs. The first system involves the housing and husbandry of amphibians under laboratory conditions such as in the case of the amphibian facility at the University of Michigan (documented in Nace 1968 and Nace *et al.*, 1996). Under this management regime, environmental conditions can be manipulated and enclosure design and quarters can be modified to produce the maximum number of metamorphs with a given amount of resources. The second

technique involves the use of artificial ponds under semi-natural conditions. The latter technique has been chosen for the leopard frog reintroduction project and offers both benefits and some limitations. Culturing of tadpoles for experimentation or other purposes is not difficult. However, the use of the current rearing ponds for the reintroduction project offer a variety of challenges because of their semi-natural conditions, resulting in a number of environmental and ecological variables that remain uncontrolled. Several environmental, biotic and abiotic factors influence tadpole ecology (Dunson and Travis 1991) and have major effects on internal and external morphology and development (McDiarmid and Altig 1999). Some of these include population density (Adolph 1931, Beebee 1991), temperature (Harkey and Semlitsch 1988), light (Edwards and Pivorun 1991), pH (Cummins 1989), dissolved oxygen (Bradford and Seymour 1988; Crowder et al., 1998) and diet (Leibovitz *et al*., 1982, Marshall *et al*., 1980). The influences of some of these factors will be discussed below.

5.1 Draw Sites

In order to collect egg masses for the 2000 rearing program, exact spawning sites were identified by night-time call surveys. Wright (1920) stated that leopard frogs may lay eggs at any time of the day, even at noon, but more frequently spawn at night. Information from biologists experienced with leopard frog natural history supported the conclusion that the majority of calling activity occurs after dark in Alberta. Hine *et al*. (1981) observed that cooler temperatures extend the period between calling and egg laying and that warm conditions compress the time. On occasion, leopard frogs may begin calling following rainy evenings, during showers or on cloudy days in the summer (Wright 1914). In Alberta, according to records held in the Biodiversity Species Observation Database (queried April 2000), egg masses have been observed between 23 April and 9 May and calling adults have been recorded between late April and early June. The indication of calling males will not guarantee the presence of egg masses because sufficient time must be allowed for females to deposit their eggs after the first male begins to call. In Wisconsin, Hine *et al*. (1981) found that females usually arrive at breeding ponds 5-7 days (occasionally 3-14 days) after male frogs, and deposit egg masses within 2-7 days after arrival. Nocturnal call surveys followed by visual surveys at locations where male frogs were heard calling the previous night remains an effective method of identifying spawning sites. However, this irregular spawning activity may result in difficulties finding egg masses if the egg masses hatch before they can be located or if they are searched for before calling and spawning begins.

Environmental conditions such as air temperature and water temperature at the draw site should be considered a priority when looking for egg masses. For example, Wright (1920) observed the phenology of leopard frogs for the latitude of Ithaca, N.Y of which may be applied to all of the northern United States. He found that leopard frogs emerge from hibernation when the water temperature reaches 5°C to 10°C. Wright (1920) also determined that leopard frogs begin to spawn when air temperatures ranged between approximately 6°C to 9°C or average approximately 10°C to 12°C. He recorded water temperatures at the onset of breeding from 6°C to 7°C and 10°C to 18°C at its peak. Hine *et al*. (1981) observed leopard frog breeding activity in Wisconsin similar to what Wright observed, and found that breeding was closely associated with water temperature and did not occur until water temperatures reached 10°C. Conversely, breeding activity was depressed and halted when air and water temperatures fell below 10°C.

During the collection of egg masses for 2000, night temperatures during call surveys ranged between 7°C to 10°C. Only a limited number of adult frogs were heard calling during the call surveys. This may suggest that a small number of individual male frogs were at the sites where calling was detected or that calling was depressed because of environmental conditions. Water temperatures were not taken during call surveys or at the location from which egg masses were collected. Detailed studies of leopard frog phenology, as it relates to water and air temperature at egg mass draw sites and other areas of Alberta would provide valuable information on the effects of temperature on leopard frog ecology.

The method of performing egg mass searches in known or suspected calling sites was found to be quite productive since egg masses are frequently deposited in a localized area within a given pond. Merrell (1977) found that egg masses were often concentrated in an area of between 1-4 m^2 area where breeding animals had congregated during calling activity (Wright 1920). Hine *et al.* (1981) also found that egg masses were grouped closely together in the part of the pond that received the greatest amount of early morning and early afternoon sunlight and possessed the shallowest water. Shallow water warms faster then deeper water and thus enables the locomotive functions of exothermic amphibians, important for breeding activity, as well as the early development egg masses and tadpoles.

These findings were supported by field observations in the spring of 2000. Although no calling activity was noted at the draw site 2, several egg masses (including remnants of hatched egg masses) were observed within a small area of a shallow portion of the pond. These finding were duplicated at the two other collection sites. Egg mass observations and evidence of calling leopard frogs were scant between the period of 4 May and 6 May when the egg masses were collected. However, it can not be determined if the calling and spawning period was concluding or just beginning. In Wisconsin, Hine *et al.* (1981) reported that egg masses appeared 5-14 days after calling began. This would suggest that calling activity in the area may have begun up to two weeks prior to the observation of egg masses at the draw sites. Successful collection of egg masses in 1999 and 2000 supports the current method of obtaining egg masses to ensure stock for the captive-rearing program of the reintroduction project.

5.2 Rearing Site

Fichter and Linder (1964) reported that *Rana pipiens* egg masses contain up to 6000 eggs. Merrell (1968) estimated that 2000-5000 eggs were produced per mass. These findings suggest that leopard frog egg masses collected in 1999 and 2000 at the designated draw sites contain considerably fewer eggs than the maximums reported by the authors noted above. The number of eggs produced by a given female may be directly related to her condition prior to hibernation and at the onset of breeding or it may reflect her age or size.

5.3 Water Quality

Little information is available on the effects of various water quality parameters on the eggs, larvae and adults of leopard frogs. Nace *et al.* (1996) recommended that water quality standards for larval and adult amphibians be held within the limits prescribed for fish. The parameters

suggested by Nace *et al.* (1996) include alkalinity and hardness ($CaCO_3$), ammonia (NH_3) and other nitrogen compounds, oxygen and pH. Others parameters include carbon dioxide, micro-organisms, PCBs (polychlorinated biphenyl compounds), fluorides, chlorine and heavy metals (Nace *et al.* 1996). A few of the above parameters as they relate to amphibians are discussed below.

Alkalinity and General Hardness

Nace et al. (1996) recommends that total alkalinity be maintained between 150 and 250 ppm. For the most part the alkalinity in both ponds remained within the recommended value suggested by Nace et al. (1996). Carbonate hardness (KH), also known as alkalinity, helps to stabilize pH and is the measure of carbonate and bicarbonate ion concentrations dissolved in water. General hardness (GH) is the measure of calcium and magnesium ion concentrations dissolved in water. Hard water ($\geq$ 200 ppm) is high in calcium and magnesium and soft water (50-100 ppm) is low in these minerals (Aquarium Pharmaceuticals Canada 2000). Correct alkalinity and hardness values in water provide tadpoles with necessary minerals during development (Nace *et al.* 1996). KH and GH values ranged between 125.3 ppm to 250.6 ppm and 161.1 ppm and 232.7 ppm, respectively in the east and west rearing ponds between 17 May and 30 August (see section 4.3).

Ammonia

Although ammonia concentrations were not measured in 2000, it has be established (Nace *et al.* 1996), that ammonia levels greater than 0.2 ppm are harmful to fish and may also be detrimental to amphibians. In an established system, ammonia levels should remain at 0 ppm (Aquarium Pharmaceuticals Canada 2000). At 4 ppm, ammonium carbonate and ammonium hydroxide are toxic to fish and can cause pH changes that further stress fish and presumably amphibians (Nace *et al.* 1996). Ammonia is continually released into systems through urine, solid wastes and other decaying organic matter. Under normal conditions in a water body a natural balance occurs and nitrifying bacteria keep ammonia in check converting it to nitrite and then to nitrate. Ammonia levels in the rearing ponds should be monitored on a regular basis in future years.

Nitrates and Nitrites

Future tests for nitrates (NO_3-) and nitrites (NO_2-) in the rearing ponds should be conducted based on recommendations made by (Nace *et al.* 1996) that these parameters not exceed 0.3 ppm. Nitrates and nitrites are produced by bacterial decomposition of ammonia and organic materials. High levels of nitrite will prevent fish from carrying on normal respiration and may be detrimental to tadpoles (Nace *et al.* 1996). In an established system, nitrite levels should be at 0 ppm (Aquarium Pharmaceuticals Canada 2000).

pH

Evidence suggests that *Rana* species develop best if the pH is approximately 6.5 and that pH levels outside the range of 6.5-8.5 may be detrimental to amphibians over extended periods (Nace *et al.*, 1996). Vatnick *et al.* (1999) found that *Rana pipiens* exhibited a 72% mortality when exposed to citrate buffer at pH 5.5 for 10 days. In comparison, a control group of *Rana*

pipiens that was held at pH 7 exhibited 3.5% mortality. Although tests were conducted on adult frogs only, other age classes including eggs and tadpoles may be similarly affected by pH values within the range tested by Vatnick *et al.* (1999). pH was recorded on an irregular basis in the rearing ponds throughout the rearing process (see section 4.3). A minimum pH of 6 was recorded on all occasions within the rearing ponds. However, it is expected that the pH in the rearing ponds may have been higher because of inaccurate interpretation of the test used.

Dissolved Oxygen

Upon hatching, tadpoles begin to breath via internal gills, pumping water through their mouths and out a small, tube-like structure located on the left side of their body (Souder 2000). Under low oxygen conditions, tadpoles are able to meet their respiratory requirements through the process of bobbing (swimming to the surface for air). To breathe air, tadpoles fill their buccal cavity with air at the water surface (McDiarmid and Altig 1999). Dissolved oxygen (D.O.) levels were not recorded in the rearing ponds during the 2000 rearing season. However, bobbing was noted, especially toward the end of metamorphosis. Critical D.O. levels for leopard frog tadpoles are largely unknown, however a study conducted by Wassersug and Seibert (1975) suggests that D.O. levels below 2 ppm and 3 ppm are not immediately lethal to leopard frog tadpoles. Nace *et al.* (1996) recommended that oxygen requirements for fish be followed for the aquatic stages of amphibians and suggested that oxygen levels not fall below 5 ppm.

5.4 Tadpole Development

Strauss and Altig (1992) stated that most of the growth of a tadpole follows the exponential phase of a sigmoid curve. An initial period of maximum growth (with minimal development) is preceded and followed by periods of significant development and little growth. Most anuran larvae are considerably flexible in their growth rates and development (Wilbur and Collins 1973) and may respond directly to environmental influences (Hays *et al.*, 1993).

The development of leopard frog embryos begins within two to three hours after the eggs are laid (Dickerson 1906). Wright (1914, 1920) found that leopard frog eggs hatched in 13 to 20 days under normal field conditions. Souder (2000) stated that leopard frog eggs took 10 days to hatch. Dickerson (1906) observed that most tadpoles hatch out of the jelly 9-10 days after being laid. In Wisconsin, Hine *et al.* (1981) found that hatching rate depended on water temperature and that tadpoles were observed in 5-9 days under constant temperatures but took up to 14 days to appear under cooler conditions. In Manitoba, hatching extended over a period of 8-18 days and occurred between the middle and the end of May (Wershler 1991). These findings suggest that it may take up to three weeks, under certain water temperatures, for leopard frog eggs to hatch.

Dickerson (1906) observed that for 2-3 days after hatching the tadpoles remain relatively inactive and that approximately 6 days after hatching (16 days after being laid) they become free-swimming and active. The tadpoles that hatched from egg masses obtained at draw site 2 were described as free-swimming and active seven days after hatching and were counted and released into the rearing pond on 26 May. The tadpoles that hatched from the egg mass collected at Bow City Burrow Pit were described as free-swimming and active 11 days after hatching and released on 30 May.

The water temperature in the rearing ponds ranged from approximately 13°C to 23 °C in the east pond and 13°C to 22 °C in the west pond over the course of the rearing period (see section 4.3). Water temperature is an integral part of the developmental process of frog eggs and tadpoles. Water temperature heavily influences tadpole activity, behaviour, growth and development, metabolic rate and rate of metamorphosis (Kollros 1961, Smith-Gill and Berven 1979, Wilbur and Collins 1973). Souder (2000) noted that tadpoles are highly responsive to temperature and under warmer conditions develop at a faster rate. Information on lethal maximum and minimum temperatures for leopard frog eggs and tadpoles is very limited. Moore (1944) found the upper limit of tolerance for leopard frog eggs from northern localities to be 28°C to 29°C and up to 34°C in southern localities. Moore also stated that eggs from southern localities were killed at 6°C but eggs to the north can still develop at that low temperature. The optimal water temperature for leopard frog development during the larval stage may be 20°C (Nace *et al.* 1996).

Metamorphosis (the process through which tadpoles grow legs and reabsorb their tail) began on 21 July (west rearing pond) and 24 July (east rearing pond) and more generally between 19 July and 26 July for both ponds. Seventy-two to 80 days elapsed between the complete hatch of the egg masses and mass emergence of young-of-the-year (YOY) frogs. Wright (1920) observed that it usually takes 60 to 80 days hatchlings to become frogs. Wershler (1991) stated that the metamorphosis period ranges from 60 to 90 days. Wendlandt and Takats (1999) found that it took 93-97 days to complete metamorphosis from hatch time. It appears that the leopard frogs reared in 2000 developed and transformed within an expected period of time based on observations made by other authors, suggesting that the environmental conditions within the rearing ponds closely match natural conditions in the wild.

Hine *et al.* (1981) found that the average weight and SVL of leopard frogs at metamorphosis over a 2 year study in Wisconsin was 2.1 g / 39 mm SVL. In 1991, Seburn (1993) calculated the average SVL of young of the year at several breeding sites in Alberta at 35-40 mm. The average weight and snout to vent length (SVL) of metamorphs (5-12 days after mass emergence) in 2000 was 5 g / 36 mm SVL (n=99) from the east rearing pond and 7 g / 41 mm SVL (n=42) from the west rearing pond. In 1999, the average weight and SVL of 178 juvenile frogs weighed and measured between 16-23 September was 6.7 g / 36 mm SVL. This limited information suggests that the size and weight of leopard frogs reared at the Raven Brood Trout Station may be comparable to leopard frogs observed in nature.

5.4.1 Tadpole survival rate

In nature there is always a considerable loss of tadpoles, particularly just before transformation (Wright 1920). Based on visual inspection of a number of egg masses, Hine *et al.* (1981) found that approximately 5% of the eggs in each mass were lost to parasitism, diseases and other factors.

A number of measures were undertaken in this project to reduce the loss of tadpoles during the rearing process and to help ensure the optimal survival rate during the various development stages of the leopard frogs in the rearing ponds. A total of 1477 juvenile leopard frogs were successfully reared in 2000 from 6687 tadpoles (counted shortly after hatching). The resulting

survival rate in 2000 of 22.1% was an improvement over the 1999 survival rate of 17.2% (1430 juvenile frogs produced from 8292 tadpoles, counted shortly after hatching). Survival rates from both years indicate that the efforts taken to ensure optimal survival rate of the developing frogs may be responsible for an unusually high survival rate during the rearing process versus what has been observed in nature. For example, Merrell (1977) estimated that 600 000 leopard frog eggs yielded 20 000 YOY (a survival rate of 3%). Hine *et al.* (1981) estimated that the survival rate of leopard frogs (egg to YOY) at a number of study ponds was 1-6% using an average of 3500 eggs per egg mass.

5.4.2 Tadpole depredation

Most of the habitats occupied by tadpoles are used by a variety of other taxa, including many that compete with tadpoles (Morin *et al.* 1988) and a number of predators (Cooke 1974, Rowe *et al.* 1994). The interactions between tadpoles and their predators have been well studied and documented in detail. Field data on survival rates of tadpoles suggests that depredation is a major source of mortality at the egg stage (McDiarmid and Altig 1999, Villa *et al.* 1982) and through metamorphosis (Arnold and Wassersug 1978, Wassersug and Sperry 1977). Studies examining the growth and survival rate of tadpoles have suggested that tadpole mortality rates decreased as tadpoles grew (McDiarmid and Altig 1999). Data from other studies showed that the mortality rate of tadpoles over the larval period usually remained constant (Petranka 1985). Herreid and Kinney (1966) supported Petranka's findings and found that populations of wood frogs in central Alaska all experienced nearly constant proportional mortality from egg deposition to transformation. Mortality rates were not monitored through the rearing period in 2000 (except at the egg stage and emergence stage). However, management steps currently undertaken should be continued to ensure that all age classes (or development stages) of the captive reared tadpoles are protected in order to maximize young of the year production.

It has generally been accepted that the bulk of predation on anurans appears to during the various stages of metamorphosis, by a variety of invertebrate and vertebrate predators. For example, Litch (1974) suggested that most tadpole mortality (*Rana aurora* and *Rana pretiosa*) was caused by predation and that the cumulative survival from egg to young frog was less than 1% and 5% at a pond site and river site, respectively. Calef (1973) found that *Rana aurora* tadpoles depredated at a relatively constant rate and that only about 5% survived to metamorphosis from a number of egg masses deposited in Marion Lake, British Columbia over two years. Conversely, when predation is limited or absent, survival rates through metamorphosis can increase (McDiarimid and Altig 1999). Mortality between egg mass and metamorph stages of development during the 2000 rearing season was approximately 78% with the aid of predator management. It should be noted that population regulation at the larval stage may also be strongly influenced by tadpole densities, as discussed below.

Leopard frog egg masses and tadpoles are vulnerable to a wide range of predators. These include water beetles (especially their larvae), dragonfly nymphs, fish, mink and even crustaceans such daphnia (Wright 1920). Fish, which are often restricted to the same habitat as frogs, are a serious predators of tadpoles (Wright 1920). During the 2000 rearing season, water tigers (predacious diving beetle larvae) and mink were identified to be the primary predators on

the tadpoles and young of the year frogs, respectively. Brodie and Formanowicz (1983) found considerable predation by larval diving beetles (*Dytiscus* sp.), whose growth patterns enable them to prey on growing tadpoles at a nearly constant rate. Avian predators such as the belted kingfisher and great blue heron were an additional threat to the tadpoles and were observed at the rearing ponds on several occasions although none were seen actually capturing tadpoles or young frogs. Fish were not present in either rearing pond and as a result posed no threat during the rearing process.

Predation levels can be influenced by the relative size of the predator and the prey (Eklov and Diehl 1994, Wilbur 1988) and in systems with size-limited predators, large body size can provide protection from predation (Bronmark and Miner 1992, Paine 1976, Persson *et al*., 1996). For example, larger tadpoles swim faster (Wassersug and Hoff 1979) and may be more capable of escaping predators (Feder 1983). Predators may also be gape limited (i.e. have jaws or mouths that are too small or weak to harm tadpoles at a given size) and therefore larger tadpoles may be afforded refuge from predators. Permanence of the waterbody also affects predation levels on tadpoles (Babbitt and Tanner 1998). For example, a number of predacious diving beetle larvae were able to over-winter in the east rearing pond in 1999/2000, resulting in an assemblage of established aquatic predators in 2000. The east rearing pond exhibited a mortality rate of 79.9%, while the west rearing pond exhibited a mortality rate of 74.1% - a difference of 5.8%. The west pond was drained prior to the introduction of the egg masses and the aquatic invertebrate predators were much fewer and smaller in size than those in the east rearing pond. It is important to note, however, that the mortality rate observed in the rearing ponds could also be attributed to tadpole density or some environmental factor.

A number of factors influence the vulnerability of tadpoles to predators, including tadpole behaviour, body size, colouration and habitat preference (McDiarmid and Altig 1999). Inter-specific predation on eggs, hatchling tadpoles and tadpoles at various stages of development may be advantageous for the population if the depredation reduces competition for food and other resources, thus increasing the body size and survival rate of the metamorphs (McDiarmid and Altig 1999). The thinning effects of depredation also result in more rapid development of the surviving tadpoles (Wilbur 1987, 1988). Therefore, the presence of predators may provide a mutualistic relationship between predators and those tadpoles that escape depredation (Babbitt and Tanner 1998). The removal of a proportion of the predator base within both rearing ponds may have increased the overall productivity of both ponds by reducing predator pressure without eliminating it entirely. Removing all predators may effect overall densities, which in turn may negatively affect tadpole growth and health.

Increased habitat complexity would likely increase productivity within both ponds by reducing depredation rates. Currently, a large proportion of the substrate along the shoreline of both rearing ponds consists of cobble. Tadpoles frequent this cobble area on sunny days because of the relativity warm and shallow water. With minimal emergent and aquatic vegetative cover, the foraging success of aquatic invertebrate and vertebrate predators is increased through ease of manoeuvrability and increased visual range (Crowder and Cooper 1982). As the two rearing ponds age they will increase in habitat complexity, however the cobble area along the shore may remain void of vegetation for sometime. In this case a management initiative should be

considered to add emergent plants to this area, thus providing a visual barrier to reduce predation rates by both aquatic predators and avian predators in this area.

5.4.3 Tadpole competition

Although not thought to be an issue in 2000, McDiarmid and Altig (1999) note that competition between the same species of tadpoles, other tadpole species, and with other phyla may influence growth rates, development and survival of tadpoles. Both adult and subadult wood frogs and boreal toads were observed in each of the two rearing ponds on several occasions. Upon observation they were immediately relocated to suitable habitat outside of the rearing pond and no egg masses, tadpoles or young were observed at anytime during the rearing process in 2000. Relyea (2000) found that wood frog tadpoles suppressed the growth of leopard frogs reared in pens and natural ponds. Past studies involving wood frog and leopard frog responses to competition indicate that the growth of the two species can vary (Werner and Glennemeier 1999). In order to avoid competition issues between species, efforts to remove foreign amphibian species from the rearing ponds should be continued.

5.4.4 Tadpole diet

Tadpoles interact with a variety of other types of herbivores, and compete with a number of herbivorous aquatic insects, crustaceans and zooplankton for food and similar resources (McDiarmid and Altig 1999). Tadpoles are specialized feeders that may ingest planktonic material from the water column, obtain organic materials from pond sediments, or scrape material from various substrates (McDiarmid and Altig 1999). Leopard frog tadpoles are primarily herbivores, however under certain circumstances may extend their diet and tend to be omnivorous as they grow.

Tadpoles may be reared on a variety of commercial and artificial foods such as rabbit pellets, trout chow, frog brittle and various fish foods (McDiarmid and Altig 1999). A number of authors suggest feeding tadpoles lightly boiled raw Romaine or escarole lettuces, or spinach. However, lettuce alone may be deficient in some of the nutrients necessary for metamorphosis (McDiarmid and Altig 1999, Nace et al. 1996). Briggs and Davidson (1942) reported that spinach produced the most rapid development and growth in leopard frog tadpoles. In contrast, Berns (1965) reported that a spinach diet might adversely affect a number of amphibian species through the development of calcium oxalate kidney stones. Nace et al. (1996) reported that tadpoles could survive when fed a diet of raw liver and powered or hard-boiled egg yolk, but warned that these types of food can reduce water quality as they decay. The addition of any type of supplementary food to the diet of growing tadpoles contained in the rearing ponds might produce complications relating to water quality. Based on field observations during the rearing period, tadpole food availability and quality did not appear to be a limiting factor during the growth and development of the tadpoles. Moreover, Dickerson (1906) stated that hunger may cause earlier degeneration and re-absorption of the tail and opercular membrane, thus decreasing the time it takes tadpoles to metamorphose. In contrast, good feeding and low temperatures may delay metamorphosis (Dickerson 1906).

Both rearing ponds were maintained at a similar water depth during the rearing process; however, because the east pond contained water throughout the winter, and early spring, it had significantly more algae present at the time of tadpole release. The east pond also had a greater number of tadpoles released into it (n=4973) compared to the west-rearing pond (n=1719). The average weight of a recently transformed leopard frog in the east rearing pond was 5 g (n=99) while the average weight of a recently transformed leopard frog in the west pond was 7 g (n=42). Overall, the ponds did not appear to be taxed for vegetative food for the tadpoles to feed, thus this difference in weight could be attributed to some effect of tadpole densities rather than food availability.

5.4.5 Tadpole density

It has been noted that over-crowding of tadpoles under laboratory conditions will produce stunted growth (Adolph 1931). Lynn and Edelman (1936) suggested that physical encounters between individual tadpoles under crowded conditions could cause stress resulting in decreased growth rates. Experiments conducted by Lynn and Edelman (1936), involving *Rana sylvatica* in aquaria, supported their hypothesis as they found that crowded tadpoles experienced higher rates of mortality and transformed later than did tadpoles under less crowded conditions. Rugh (1934) found similar results in an experiment on *Rana pipiens*. And Gromko *et al*. (1973) suggested that adrenocortical responses caused by social stress reduced growth in larval *Rana pipiens* when reared under crowded conditions.

Wilbur (1976) studied the effects of density on the survival, growth and development of *Rana sylvatica* tadpoles and found that the mean body mass at metamorphosis decreased exponentially with density. Adolph (1931), found that both *Rana sylvatica* and *Rana pipiens* growth rates declined with increasing density. He also found that growth rates declined as the size of the rearing container decreased. Adolph (1931) attributed these effects to stress caused by over-crowding, which decreased growth efficiency and increased the metabolic rate of the tadpoles. Under conditions with low tadpole density and high resource availability most tadpoles will grow at greater than average rate and will not metamorphose until they reach maximum size. In contrast, at higher tadpole densities and lower resource availability, tadpoles grow slowly and metamorphose at or near minimum size (McDiarmid and Altig 1999). Observations made in 2000 can be weakly correlated with these findings, but it should be noted that the effects of food availability and predator interactions are not fully understood. The tadpoles in the east pond morphed at a smaller size (average SVL of 36 mm and average weight of 5 g) then to the frogs in the west pond, which morphed at an average SVL of 41 mm and average weight of 7 g. The density of frogs in the east pond was higher than that of the west pond (4968 tadpoles in the east pond versus 1719 tadpoles in the west pond and the tadpoles in the west pond began to metamorphose and emerge three days prior to the east pond.

Growth inhibition is common in laboratory populations of *Rana pipiens* tadpoles. Although it may not occur in natural populations on a large spatial scale, it may be more important where tadpoles are locally dense, such as small ponds or hatchling aggregations (McDiarmid and Altig 1999). John and Fenster (1975) suggested that spatial complexity might be important in controlling *Rana pipiens* tadpole growth rates and that increasing spatial complexity would reduce stress on tadpoles by decreasing the encounter rate between tadpoles. In experiments

involving *Rana pipiens* tadpoles, John and Fenster (1975) found that the average body volumes of tadpoles at metamorphosis in bare aquaria were significantly lower than those of tadpoles contained in aquaria fitted with partitions that partially subdivided the aquaria into connected chambers. John and Fusaro (1981) found that the size of the growth chambers could affect the growth rates of larval *Rana pipiens*, fed ad labium. Overall, the rearing ponds offered a variety of cover in the form of cattails, various sedges and grasses and floating vegetation. Whether this cover is sufficient to afford visual barriers and the degree of habitat complexity required for optimal growth rates and metamorph size is not known but should be maintained.

Several authors have also suggested that living cells also limit the growth rate of tadpoles (McDiarmid and Altig 1999). For example, Richards (1958) found that the growth rates of *Rana pipiens* tadpoles were reduced in water conditioned by larger *Rana pipiens* tadpoles. Similarly, Bardach et al. (1972) found that individual tadpoles that grew more rapidly and obtained a larger size released a growth-inhibiting substance that affected smaller tadpoles, resulting in longer metamorphosis period. This drawn out emergence period may benefit juvenile frogs by averting mass predation or unfavourable terrestrial conditions at a particular time (Bardach et al., 1972). The emergence period in 2000 extended from 19 July to 3 August with a small number of individuals emerging later than 3 August and was believed to be normal.

Research and literature information on a prescribed *Rana pipiens* tadpole density, supportive of optimal growth and development, is limited. Nace *et al*. (1996) suggest that when feeding begins, after hatching, *Rana pipiens* larvae should be thinned to 50 or fewer per litre and to 4 to 6 tadpoles/litre near metamorphosis. The rearing ponds, were estimated to contain 292 600 L (77 300 gallons) and 339 600 L (89 710 gallons) of water in the east and west pond, respectively. These calculations were based on maximum obtainable depth in each rearing pond. Tadpole densities calculated for the east and west rearing pond, after the hatchling tadpoles were released from the predator exclosures, were 0.017 tadpoles/litre and 0.005 tadpoles/litre, respectively. Densities near metamorphosis (numbers determined based on actual number of metamorphs counted and marked) were 0.004 tadpoles/litre for the east rearing pond and 0.001 tadpoles/litre for the west pond. Provided that there is enough cover or visual barriers, these results suggest that tadpole densities may not be a limiting factor for optimal growth and development in the rearing ponds.

5.5 Release

A reintroduction is a success if it results in a self-sustaining population (Griffith *et al*. 1989). Theoretical considerations predict that population persistence is more likely when the number of founders is large (Griffith *et al*. 1989). The persistence of the founding population may be enhanced if the rate of population increase is high and the effect of competition low (Griffith *et al*. 1989).

It is imperative that tadpoles for the reintroduction project are captive-reared under optimal conditions because these conditions affect growth, development and survival rate after metamorphosis. As discussed, these conditions include food availability and quality, water temperature, larval density, depredation, competition and water quality. Optimal conditions can result in larval amphibians transforming at a larger size, which may allow them to have better

physiological and locomotive performance once in a terrestrial environment (Pough and Kamel 1984, Goater *et al*. 1993), thus increasing their survival rate. Larger size at metamorphosis may also lead to earlier first reproduction and larger size at first breeding (Berven 1990, Berven and Gill 1983, Smith 1987, Semlitsch *et al*. 1988).

6.0 MANAGEMENT IMPLICATIONS AND FUTURE DIRECTION

Management Implications

1. The collection of egg masses must coincide with local ambient temperatures at the draw site rather then the calendar day. A number of authors have reported breeding activity begins when water and air temperatures reach approximately 10°C. In addition, consideration should be taken that female leopard frogs generally arrive at breeding ponds after males and do not immediately deposit egg masses.

2. To help ensure greater success when using nocturnal call surveys to identify leopard frog breeding sites, surveys should be conducted when air and water temperatures are within the prescribed range supportive of breeding activity. Call surveys should also be conducted after dark and at previously determined areas of a given waterbody that possess shallow water and possibly emergent vegetation.

3. Additional water quality parameters should be tested at egg mass draw sites and during rearing process. Parameters suggested include alkalinity and hardness, ammonia, nitrate, nitrite, phosphate, pH, dissolved oxygen and water temperature.

4. The number of egg masses collected for the captive rearing program should be increased to provide more tadpoles for captive rearing and potentially resulting in a greater number of young frogs for reintroduction. An increased load of tadpoles in the rearing ponds may also provide insight in the carrying capacity of the rearing ponds relating to the emergence size and health of metamorphosed frogs.

Future Direction

The idea of a leopard frog captive-breeding program, involving the Calgary Zoo, should be investigated. This potential program would involve a group of leopard frogs, supplied by the current reintroduction program at the Raven Brood Trout Station, to be held at the Calgary Zoo. One of the primary goals of such as program would be to maintain a genetically viable breeding population of leopard frogs over the long-term and that could be used to produce animals for the purpose of reintroduction.

A number of areas of research would arise from a captive population of leopard frogs at the Calgary Zoo. Areas of research could include hibernation (role of air and water temperature, photoperiod and other water quality conditions during hibernation), reproduction (how weight loss and water quality conditions during hibernation and weight gain prior to hibernation affect reproductive success) and the evaluation of leopard frog genetics and the role genetics play on survival rate during hibernation and reproductive success. This research would provide information that could be used to better manage and benefit northern leopard frogs in the wild. Finally, through existing education and public programming at the Zoo, a number of education and public awareness opportunities relating to the conservation of amphibians, the leopard frog reintroduction project and the leopard frog would be possible.

New sites for future leopard frog releases should be identified in the upper headwaters of the North Saskatchewan and Red Deer River drainages. Priority should be given to finding new sites in the headwaters of the North Saskatchewan drainage in accordance to the projects objectives. Chosen sites must possess ecological criteria supportive of leopard frog populations, such as appropriate under-ice dissolved oxygen and temperature levels, and suitable breeding and summering habitat. Ideally, sites with breeding habitat void of predatory game fish should be selected. Future sites should also possess long-term preservation or conservation protection and experience minimal disturbance or habitat degradation. Also, new release sites that possess some degree of public access, such as sites associated with provincial parks, interpretative centres, etc. would be valuable in the enhancement of public education and the monitoring of released frogs.

New release sites, when first investigated, should be done so during the summer to ensure the proper assessment of breeding and summering habitat. The true measure of these habitat attributes can only be accomplished during this time. For example, snow cover would mask the health and physical structure of summer habitat, and the permanence of breeding habitat can only be confirmed by a late summer visit. Although wintering habitat is of paramount importance for a potential release site, this habitat attribute can be estimated at potential release site initially, during the summer visit. A relatively good judgement on the quality and potential of wintering habitat can be made based on depth of water, flow rate (if present), plant communities, presence of fish and previously documented information or knowledge on the waterbody in question. Once suitable breeding and summering habitat at a potential release site is confirmed, follow up surveys for suitable winter water quality for the successful hibernation of leopard frogs, should be conducted.

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Appendix A Number of frogs captured from the west and east-rearing pond, color each was marked and site at which they were released.

Date	Number of frogs collected		Color marked	Total Released		
	East	West		Cooling Pond	Oxbow	West Rearing Pond
08 August	331	-	Green	331	-	-
09 August	230	-	Green	230	-	-
09 August	222	-	Orange	-	222	-
14 August	-	358	Orange	-	358	-
14 August	20	-	Orange	-	20	-
14 August	30	9	Green	21	-	-
15 August	-	70	Yellow	-	11	70
15 August	130	-	Yellow	-	-	130
16 August	-	7	Green	7	-	-
16 August	1	-	Green	1	-	-
16 August	9	-	Orange	-	9	-
22 August	8	-	Green	8	-	-
23 August	16	-	Green	16	-	-
01 September	11	-	Green	11	-	-
08 September	4	-	Orange	-	11	-
12 September	9	-	Green	9	-	-
13 September	11	1	Green	12	-	-
Total	1032	445	Total	646	631	200
Grand Total	1477		Grand Total	1477		

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